

VERIFICATION OF TRANSLATION

Re: JAPANESE PATENT APPLICATION NO. 1997-214604

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hereby declare that I am the translator of the
document attached and certify that the following is
true translation to the best of my knowledge and
belief.

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Dated this 13th day of February, 2002

[Document Name] Specification

[Title of the Invention] ISOFLAVONE-CONTAINING COMPOSITION

[Claims]

[Claim 1] An isoflavone-containing composition comprising
5 a isoflavone and a strain of microorganism capable of
metabolizing daidzein to equol.

[Claim 2] The isoflavone-containing composition according
to Claim 1 wherein the strain of microorganism capable of
metabolizing daidzein to equol is at least one member
10 selected from the group consisting of Bacteroides ovatus,
Streptococcus intermedius and Streptococcus constellatus.

[Claim 3] The isoflavone-containing composition according
to Claim 1 or 2 which further contains at least one
component that favors the maintenance and growth of the
15 strain of microorganism capable of metabolizing daidzein
to equol.

[Claim 4] The isoflavone-containing composition according
to Claim 3 wherein the component that favors the
maintenance and growth of the strain of microorganism
20 capable of metabolizing daidzein to equol is at least one
substance selected from the group consisting of lacto-
oligosaccharide, soya oligosaccharide, lactulose, lactitol
and fructo-oligosaccharide.

[Claim 5] An equol-containing composition comprising equol
25 obtained by causing a strain of microorganism capable of

metabolizing daidzein to equol to act upon a isoflavone.

[Claim 6] The composition according to Claims 1-4 wherein
the food form is selected from the group consisting of
drinks, dairy products, fermented milk, bars, granules,
5 powders, capsules and tablets.

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

The present invention relates to an isoflavone-
10 containing composition and equal-containing composition,
and more particularly to a novel composition either
comprising blending an isoflavone such as diadzein,
genistein, daidzin or genistin with a specific strain of
microorganism or comprising equol, which composition is
15 useful for the prevention and palliation of unidentified
clinical symptoms in middle-aged to elderly women,
inclusive of menopausal syndrome.

[0002]

[Prior Art]

20 The documented collaborative research of
National Cancer Center of Japan and Helsinki University
(Finland) attributes the low incidence of gender-specific
carcinomas such as carcinoma of the prostate in men and
carcinoma of the breast or ovary in women among the
25 Japanese as compared with the European and American people

to the greater intake by the Japanese of soybean-derived foods and the consequent well-coordinated balance of hormones (H. Adlercreutz, et al., (1992) Lancet, 339, 1233; H. Adlercreutz, et al., (1992) Lancet, 342, 1209-
5 1210).

[0003]

Recently, there has been a mounting interest in the fact that isoflavones have estrogen (female hormone)-like activity (A. Molteni, et al., (1995) J. Nutr., 125, 751S-756S), and it has been reported that these compounds
10 are effective in osteoporosis which develops after the menopause when estrogen secretions have subsided or ceased (D. Agnusdei, et al., (1995) Bone and Mineral, 19 (Supple), S43-S48) as well as in menopausal syndrome (D. D. Baird,
15 et al., (1995) J. Clin. Endocrinol, Metab., 80, 1685-1690; A. L. Murkies, et al., (1995) Maturitas., 21, 195-198).

[0004]

According to the result of a survey undertaken by Margaret Lock (M. Lock, et al., (1988) Maturitas., 10,
20 317-332), the incidence of climacteric symptoms among the Japanese women is extremely low as compared with the Canadian counterparts. Based on the report, H. Adlercreutz and coworkers conjecture that the Japanese women ingest large amounts of processed soybean foods such
25 as tofu, miso, soy sauce, etc. and, hence, the plant

estrogens (isoflavones) contained in those foods are responsible for the low incidence of menopausal symptoms.

The same authors further report that, comparing the urinary excretions (24-hour urine) which are known to

5 reflect the amount of absorption of isoflavones actually ingested, the urinary excretions in the Japanese women are tens of times as high compared with the Western women (C. Herman, et al., (1995), J. Nutr., 125, 757S-770S).

[0005]

10 It is, thus, considered that the intake of isoflavones is effective for the palliation and prevention of postmenopausal osteoporosis and menopausal syndrome. Particularly, the postmenopausal life expectancy in women has reportedly increased to more than 30 years owing to
15 the recent trend toward longevity and the alleviation and prevention of various diseases and symptoms which may develop after the menopause have important meanings in that they would lead to improvements in quality of life (QOL).

20 [0006]

However, the above report, i.e. the survey report on the amount of intake of isoflavones and the urinary excretions of isoflavones in the middle-aged to elderly women in Japan reflects the results generated in a
25 limited rural area and no substantive information is

available. Moreover, the correlation between the onset of menopausal syndrome and the amount of intake of isoflavones has not been squarely analyzed and revealed.
[0007]

5 [Problem to be Solved by the Invention]

Therefore, the object of the present invention is to provide a novel composition which is effective for the prevention and palliation of the unidentified clinical symptoms in middle-aged to elderly women including the
10 menopausal syndrome, for which no effective means of prophylaxis or relief has been available.
[0008]

To accomplish the above object, the inventors of the present invention first conducted a diet or food menu
15 survey, determination of urinary excretions of isoflavones, and a questionnaire survey about menopausal syndrome (unidentified clinical symptoms) in perimenopausal women in a broad geographical area including urban communities. According to the results of the above surveys conducted in
20 116 women aged between 40 and 60 who belonged to Fukuoka Prefectural Dietitians Association, the average amounts of intake of isoflavones were 9 mg/day for daidzein and 13 mg/day for genistein. The average urinary excretions of isoflavones were 19.6 μ mol/day for daidzein, 10.0 μ mol/day
25 for genistein, and 11.9 μ mol/day for equol (mean of

subjects in whom it was detected). Incidentally, although daidzein and genistein were detected in all the subjects, equol was detected only in 46 (51.6%) of the 95 subjects.
[0009]

5 Furthermore, women with perimenopausal and those
within 5 years of the menopause being taken together as
menopausal subjects, a questionnaire survey was conducted
using 17 items which are in routine use in the diagnosis
of menopausal syndrome and the simplified menopausal index
10 (SMI) was calculated. With taking subjects with SMI
values not less than 20 as a group of high climacteric
symptoms and those with SMI values not greater than 19 as
a group of low climacteric symptoms, the amount of intake
of isoflavones and the urinary excretion of isoflavones
15 were respectively compared between the groups. As a
result, whereas no intergroup difference was found in the
amount of intake of daidzein, the amount of intake of
genistein tended to be lower in the group of high
climacteric symptoms at $p = 0.0643$. With regard to the
20 urinary excretions of isoflavones, no intergroup
difference was found with respect to daidzein and
genistein, but the excretions of equol were significantly
low ($p < 0.01$) in the group of high climacteric symptoms.
[0010]

25 The above results led the present inventors to

the finding that unidentified clinical symptoms in menopausal women are more closely related to the amount of intake of genistein and the urinary excretion of equal.

[0011]

5 In the past the relationship between the amounts of intake and urinary excretion of isoflavones and their physiological effect has been discussed without regard to specific kinds of isoflavones such as daidzein and genistein but the results of the survey conducted by the present inventors in the Japanese middle-aged and elderly women made it clear that not only the amounts of intake and urinary excretion of isoflavones in general but also the amount of intake of genistein and the urinary excretion of equol relate to the physiological effect of isoflavones, and that the rate of metabolic conversion of daidzein to equol is also closely related to unidentified clinical climacteric symptoms in menopausal women.

[0012]

20 In another study undertaken by the inventors in healthy adult volunteers (25~33 years of age), it was found that the urinary excretions of isoflavones (daidzein and genistein) after single ingestion of soy milk are increased in a dose-related fashion, whereas in subjects who showed no urinary excretion of equol, equol was not detected in the urine even when the amount of intake of

25

soy milk was increased two-fold, indicating the existence of individual difference in the metabolic pathway from daidzein to equol.

[0013]

5 Based on the above findings the present
inventors conducted further research and, as a result,
developed a novel composition which comprises equol which
is not detected in processed soybean products nor is it
taken into the body from foods in ordinary diets and a
10 novel composition which comprises a strain of
microorganism capable of metabolizing daidzein to equol
and isoflavones such as daidzein in combination. The
inventors then discovered that the intake of whichever of
the above compositions is effective in the prevention and
15 palliation of unidentified clinical symptoms in middle-
aged and elderly women and have accordingly succeeded in
providing a composition to meet the object.

[0014]

[Means for Solving the Problem]

20 The present invention provides an isoflavone-
containing composition which is characterized by
comprising an isoflavone and a strain of microorganism
capable of metabolizing daidzein to equol.

[0015]

25 According to the present invention, there can be

provided the isoflavone-containing composition wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of *Bacteroides ovatus*, *Streptococcus intermedius* and *Streptococcus constellatus*; the isoflavone-containing composition which further contains at least one component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol; the isoflavone-containing composition wherein the component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol is at least one substance selected from the group consisting of lacto-oligosaccharide, soya oligosaccharide, lactulose, lactitol and fructo-oligosaccharide; and the isoflavone-containing composition wherein the food form is selected from the group consisting of drinks, dairy products, fermented milk, bars, granules, powders, capsules and tablets.

[0016]

20 The present invention further provides an equol-containing composition which is characterized by comprising equol obtained by causing a strain of microorganism capable of metabolizing daidzein to equol to act upon a isoflavone, whose the food form is selected
25 from the group consisting of drinks, dairy products,

fermented milk, bars, granules, powders, capsules and tablets.

[0017]

[Mode for Carrying out the Invention]

5 In the present invention, isoflavones derived from soybean, kudzu, their processed products and their fermentation products can be used as one ingredient (i.e. soya isoflavones) to construct the composition of the invention, but the isoflavones contained in plants such as
10 red clove and alfalfa can also be used. The processed products include, for example, tofu, soy sauce, abura-age, soy milk and the like and the fermentation products include natto, miso, tempeh and the like.

[0018]

15 In the isoflavone-containing composition of the invention, a strain of microorganism having an ability (metabolic activity) to produce equol from daidzein is used as the other ingredient. The microorganism mentioned above includes those belonging to Bacteroides ovatus,
20 Streptococcus intermedius, and Streptococcus constellatus. Particularly preferred among such microorganisms are Bacteroides E-23-15 (FERM P-16312), Streptococcus E-23-17 (FERM P-16313) and Streptococcus A6G-225 (FERM P-16314), all of which were isolated from human stools and deposited
25 for accession by the present inventors.

[0019]

The above strain of microorganism may generally be the live microorganism as such. However, it is not limited thereto but includes its culture, a crude or
5 purified product thereof, and their lyophilizates. Its proportion is not particularly restricted but can be judiciously selected according to the kind of microorganism, among other factors. For example, in the case of *Streptococcus intermedius* in fermented milk, the
10 bacterial count is preferably controlled within the range of about $10^8 \sim 10^9$ cells/mol. The bacterial count is determined by inoculating an agar medium with a diluted sample, incubating the inoculated medium anaerobically at 37°C and counting the colonies formed.

15 [0020]

The composition of the invention using the microorganisms capable of metabolizing daidzein to equol further preferably contains a nutrient component particularly suited to the maintenance and growth of the
20 particular strain of microorganism. The nutrient component includes but is not limited to lacto-oligosaccharide, soya oligosaccharide, lactulose, lactitol, fructo-oligosaccharide, and the like. The formulating amount of such nutrients is not particularly restricted
25 but generally is preferably selected from the range of

about 5~10 weight % based on the total composition of the invention.

[0021]

The composition of the invention is generally
5 prepared by blending predetermined amounts of said
ingredients and processing the mixture into a suitable
form such as drinks, dairy products, fermented milk, bars,
granules, powders, capsules, tablets and tablets. The
blending ratio of isoflavones and specific strain of
10 microorganism is not particularly critical. However, it
is preferred in general to blend not less than 10 mg of
isoflavone as genistein, 1~5 g of oligosaccharide and
 $10^9 \sim 10^{10}$ cells (as viable cells) of microorganisms.

[0022]

15 Since the isoflavone-containing composition of
the invention contains a strain of microorganism
(primarily live cells) as mentioned above, the composition
preferably should not be subjected to heating and/or
pressurization in the course of processing into final
20 products. Therefore, in processing the composition of the
invention into such forms as bars, granules, powders,
tablets, etc., it is preferable to add the microorganism
as lyophilized cells as such or lyophilized cells coated
with a suitable coating agent.

25 [0023]

Examples of other materials which may be blended to the composition of the invention include calcium, vitamin D, vitamin C, vitamin E and the like.

[0024]

5 Further, according to the present invention, an equol-containing composition comprising equol obtainable by causing a strain of microorganism capable of utilizing daidzein to produce equol to act upon a isoflavone, especially upon isoflavones derived from soya bean, kudzu
10 and their processed food or fermented food is provided in the form of drinks, dairy products, fermented milk, bars, granules, powders, capsules, tablets and tablets.

[0025]

 The equol-containing composition of the
15 invention is very safe because the active ingredient thereof is a native substance as mentioned above and also advantageous in that it can be obtained in high yield and at low production cost.

[0026]

20 Here, equol is prepared by fermentation of the isoflavones derived from soya isoflavone, kudzu, red clove and alfalfa, preferably food materials containing soya isoflavone utilizing the microorganisms. Examples of the food materials include tofu, soy milk, soya beans, soya
25 bean embryo extracts and the like.

[0027]

More particularly, the preparation of equol from isoflavone comprises sterilizing the food materials in solution form, adding the predetermined strain of
5 microorganism thereto, and incubating the mixture at 37°C either under anaerobic conditions or under aerobic stationary conditions for about 24-48 hours to let fermentation proceed [where necessary, a pH control agent, a reducing substance (e.g. yeast extract, vitamin K1) can
10 be added].

[0029]

The form mentioned above includes but is not limited to drinks, milk products, fermented milk, bars, granules, powders, capsules, and tablets.

15 [0030]

The amount of equol in the resulting composition of the invention may preferably be about 10~50 mg based on 100 g of the total composition.

[0031]

20 The amount of intake of the composition of the invention is not particularly restricted but can be generally selected so that the urinary excretions of equol after ingestion of the composition will not be less than 5 μ M/day.

25 [0032]

[Example]

For a further detailed description of the invention, examples of preparation of foods of the invention, however, it is to be understood that the scope
5 of the invention is by no means delimited by those specific examples.

[0033]

[Example 1] Preparation of a drink

The ingredients according to the following
10 recipe were weighed and blended to provide the composition of the invention in the form of a beverage.

[0034]

| | | |
|----|-------------------------------------|--------|
| | water-soluble soybean protein | 10 ml |
| | Lacto-oligosaccharide (55% content) | 10.0 g |
| 15 | Vitamins & minerals | q.s. |
| | Flavor | q.s. |
| | Water | q.s. |
| | Total | 150 ml |

[0035]

20 [Example 2] Preparation of a fermented milk

The ingredients according to the following recipe were weighed and blended to provide the isoflavone-containing composition of the invention in the form of fermented milk.

25 [0036]

| | | |
|---|--------------------------------------|--------|
| | Water-soluble soybean protein | 2.2 g |
| | Lacto-oligosaccharide (55% content) | 10.0 g |
| | Streptococcus A6G-225-fermented milk | 100 ml |
| | Vitamins & minerals | q.s. |
| 5 | Flavor | q.s. |
| | Water | q.s. |
| | Total | 50 ml |

The Streptococcus·intermedius fermented milk was prepared by adding 10^8 cells of Streptococcus·intermedius (FERM P-16313) to 1 liter of milk and incubating the mixture at 37°C for 24 hours.

[0037]

[Example 3] Preparation of a fermented soy milk lyophilizate

15 Using 1 ml of a suspension of about 10^7 cells/ml of Streptococcus·intermedius (FERM P-16313), 100 g of soy milk was caused to undergo lactic acid fermentation at 37°C for 24 hours to provide equol. This product was lyophilized. The equol content of this freeze-dried powder was 0.1~0.3 weight %.

[0038]

The above powder and other ingredients according to the following recipe were weighed and blended to provide the composition of the invention in the form of a fermented soy milk lyophilizate.

[0039]

| | | |
|---|---------------------------------|-------|
| | Fermented soy milk lyophilizate | 2.2 g |
| | Excipient | q.s. |
| | Vitamins & minerals | q.s. |
| 5 | Flavor | q.s. |
| | Total | 20 g |

As the excipient, 17 g of cornstarch was used.

[0040]

[Example 4] Preparation of powders

10 The ingredients according to the following
recipe were weighed and blended to provide the composition
of the invention in powdery form.

[0041]

| | | |
|----|-------------------------------------|--------|
| | Crude soya isoflavone powder | 4.1 g |
| 15 | Lacto-oligosaccharide (55% content) | 10.0 g |
| | Streptococcus E-23-17 lyophilizate | 1.0 g |
| | Vitamins & minerals | q.s. |
| | Flavor | q.s. |
| | Total | 20 g |

20 The Streptococcus·intermedius lyophilizate was
prepared by growing Streptococcus·intermedius fermented
milk of Example 2 or Streptococcus·intermedius (FERM P-
16313) in a suitable liquid growth medium (GAM broth)
(37°C, 24~48 hours) and then lyophilizing the resulting
25 culture. The bacterial cell content of this freeze-dried

powder was $10^9 \sim 10^{10}$ cells/g.

[0042]

[Example 5] Preparation of granules

The ingredients according to the following
5 recipe were weighed and blended to provide the composition
of the invention in granular form.

[0043]

| | | |
|----|-------------------------------------|--------|
| | Crude soya isoflavone powder | 4.1 g |
| | Lacto-oligosaccharide (55% content) | 10.0 g |
| 10 | Streptococcus E-23-17 lyophilizate | 1.0 g |
| | Sorbitol | q.s. |
| | Vitamins & minerals | q.s. |
| | Flavor | q.s. |
| | Total | 20 g |

15 As the Streptococcus intermedius lyophilizate,
the same freeze-dried powder as in Example 4 was used.

[Document Name] Abstract

[Abstract]

[Object] This invention provides a novel composition which is effective in the prevention and palliation of
5 unidentified clinical symptoms inclusive of menopausal syndrome in middle-aged to elderly women for which no effective means of prophylaxis or relief has heretofore been available.

[Method for Achieving the Object] An isoflavone-containing
10 composition comprising isoflavone in combination with a strain of microorganism capable of metabolizing daidzein to equol or an equol-containing composition comprising equol.

[Selected Figure] None